



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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SEP 11 2002  
TECH CENTER 1600/2900

Attorney Docket No.: BERN-0040  
Inventors: Eric F. Bernstein  
Serial No.: 09/913,697  
Filing Date: January 28, 2002  
Examiner: Dodson, Shelley A.  
Group Art Unit: 1616  
Title: Compositions and Methods for Prevention  
of Photoaging

DECLARATION UNDER RULE § 1.131

I, Eric F. Bernstein, hereby declare that:

1. I am a co-inventor of the above-identified application and am most familiar with the subject matter of this application and the research effort occurring in my laboratory located in Philadelphia, Pennsylvania which lead to the discovery of this invention.

2. As evidenced by pages from laboratory notebooks attached hereto, experiments have been performed demonstrating the ability of serine protease inhibitors to protect against cutaneous photodamage in a transgenic mouse model containing the human elastin promoter linked to a chloramphenicol acetyltransferase (CAT) reporter gene. This model is well-accepted for its utility in testing compounds that may inhibit cutaneous photodamage in humans.

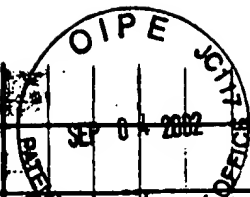
3. The experiments described in the attached laboratory notebook pages were performed under my direct supervision prior to November 5, 1998.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may

jeopardize the validity of the application or any patent issuing thereon.

9/3/02  
Date

E F Bernstein  
Eric F. Bernstein, M.D.



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Delivered sample from Tina Eckhard

TECH CENTER (Casper)  
Store 7A (Casper)

- 2 x 50ml conical tubes - T-410 D-161 (10-31-97)
- 2 x 50ml conical tubes - 472-95 D-174 (10-31-97)
- 1 x 15ml conical tube - Alpha-1-Proteinase Inhibitor  
6.8 mg/ml 472-78-8082

Mix Vitamin D milk with Aquaphor - (from clinic)  
Lubriderm - Fragrance Free  
Propylene Glycol - Sigma P-1009 (500ml)

Lubriderm : Milk	Aquaphor : Milk	Propylene Glycol : Milk
1:1 ✓	1:1 x	1:1 x
	1:2 x	1:2 x
	1:3 x	1:3 x
	1:4 x	1:4 ?
	1:5 x	1:5 ?
		1:6 ✓

Heating in 65°C bath for 10-15 min helps to dissolve, but  
Aquaphor tends to solidify. Propylene Glycol did not  
get into skin very easy until 1:5, 1:6. Lubriderm @ 1:1  
with milk = No problem

2 litter of pups - born 4 Nov 97 - Use 2mg/cm<sup>2</sup> of 1:1  
mixture of Lubriderm + D-161 and Lubriderm + D-174

$\frac{3}{4}'' = 0.2813 \text{ in}^2 = 0.7144 \text{ cm}^2$

$2 \text{ mg/cm}^2 \text{ FD } \frac{2 \text{ mg}}{\text{cm}^2} \cdot 0.7144 \text{ cm}^2 = 1.4288 \text{ mg}$   
 $= 0.0014 \text{ g}$

10 MED UNB = 35m56s

20 MED UNB = 1h11m51s

- 1 } untreated controls (cut tail)
- 2 }
- 3 } untreated UNB only (nothing)
- 4 }
- 5 → UNB + D-161 (FR)
- 6 → UNB + D-174 (BL)

- 1 } untreated controls (cut tail)
- 2 }
- 3 } untreated UNB only (nothing)
- 4 }
- 5 → UNB + Lubriderm (F.L)
- 6 → UNB + D-161 (F.R)

h-1-Proteinase 6.8 mg/ml 472-78-8082 (in PBS) 195

- 2. Cont
  - 2. UVB (43 sec ~ 55 mJ/cm<sup>2</sup>)
  - 2. 10 μM + UVB
  - 2. 20 μM + UVB
  - 2. 40 μM + UVB
  - 2. 40 μM only
- incubate for 15 min prior to exposure.
- 43 sec UVB bulbs

$$MW = \sim 52,000 \text{ g/mol}$$

$$6.8 \text{ mg} = 1000 \text{ ml} \cdot \frac{1 \text{ g}}{1000 \text{ mg}} = 6.8 \text{ g} \cdot \frac{\text{mol}}{52,000 \text{ g}} = 0.0001307 \text{ mol (M)}$$

$$= 0.1307 \text{ mmol (mM)}$$

$$= 130.7 \text{ } \mu\text{mol (}\mu\text{M)}$$

~~6.8 mg = 130.7 μM~~

$$6.8 \frac{\text{mg}}{\text{ml}} = 130.7 \mu\text{M}$$

$$\left[ \frac{2.081 \text{ mg}}{\text{ml}} = 40 \mu\text{M} \right]$$

$$\left[ \frac{1.04055 \text{ mg}}{\text{ml}} = 20 \mu\text{M} \right]$$

$$\left[ \frac{0.52028 \text{ mg}}{\text{ml}} = 10 \mu\text{M} \right]$$

33 dishes (3ml/dish) = 99 ml

3 dishes (3ml/dish) = 9 ml

3 dishes (3ml/dish) = 9 ml

68.673 mg 10.0

6.8 mg

So, take 10.099 ml of 6.8 mg = 68.673 mg

+ 22.401 ml of PBS = 40 μM conc. (33 ml total volume)

Pre incubate 4 dishes w/ 3ml for 12 min (15 min total) = 12 ml

"Shine" 4 dishes w/ 3ml for 43 sec = 12 ml

24 ml

9 ml Remain @ 40 μM conc.

+ Another 9 ml of PBS = 18 ml total = 20 μM

Pre incubate 2 dishes w/ 3ml = 6 ml

"Shine" 2 dishes w/ 3ml for 43 sec = 6 ml

12 ml

51392 -  
 Anti-Typhoid ~ 54000 Ia

$$52,000 \frac{g}{mol} \times \frac{(mol)}{1000mmol} \times \frac{1mmol}{1000\mu mol} \times \frac{1000\mu mol}{L} \times \frac{L}{1000ml} \times 40ml = 2.08g$$

1) Cont

2)

3) WUB (43.1)

4)

5) 0.01  $\mu M$  } UV  
 6) 0.1 } +435 Incub.  
 7) 1 } 5m.  
 8) 10

9) 0.01  $\mu M$  } UV  
 10) 0.1 } +435 Incub.  
 11) 1 } 15m.  
 12) 10

10  $\mu l$  anti

	pt.	Tris
1	0.2920	36.8
2	0.2750	60.4
3	0.1660	100 $\mu l$
4	0.1770	93.8
5	0.2100	79.0
6	0.2270	73.1
7	0.2360	70.3
8	0.2180	76.1
9	0.2420	68.6
10	0.1910	86.9
11	0.2250	73.8
12	0.2030	81.8

